METHOD OF INTRAVITAL LUMINESCENCE MICROSCOPY OF THE LIVER

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An apparatus is suggested for intravital luminescence microscopy of the liver (and also of other viscera) in mammals. It consists of a series of independent units: an optical unit for making visual observations and photographic recordings, a device for fixing the animal, incorporating a controlled electric heater, and a unit for irrigating the test object with a measured volume of fluid at an assigned temperature. The method described permits observations to be made on the state of the liver for very long periods of time (6-8 h), while maintaining the conditions as close to physiological as possible.

An important problem in the study of the microcirculation is investigation of the anatomy, physiology, and pathology of the viscera, including the liver, by means of biomicroscopy.

Development of techniques of intravital microscopy of the liver has proceeded in two ways: 1) biomicroscopy of the liver by means of light-conducting methods [1, 6, 7], and 2) intravital luminescence microscopy [2-5].

With the need for studying the microcirculation of the liver and details of its changes in experimental hepatitis, a comparative study was made of methods of investigating the state of the liver in vivo. Attention was concentrated on luminescence microscopy.

The first attempt at intravital luminescence microscopy was made in 1928, on the choroid of the living eye [4]. This method was later improved substantially by Ellinger and Hirt [2, 3], and used to study various mammalian organs. Considerable progress in this field was achieved by Hanzon [5]. His method of intravital investigation of the liver is the best both technically and in principle.

The basic principles of Hanzon's method were also used by the present writers. They are to create and maintain conditions as close to physiological as possible throughout the very long duration of the experiment (6-8 h). A number of technical advances have been incorporated, considerably simplifying Hanzon's method.

The proposed appratus for intravital luminescence study of organs of small and medium-sized mammals consists of a series of independent units: 1) an optical unit for making visual observations and photographic recordings; 2) a device for fixing the animal, incorporating a controlled electric heater; and 3) systems for irrigating the test object with a measured volume of fluid at an assigned temperature.

The Soviet ML-2 luminescence microscope and contact objectives giving different magnifications (10×, 25×, 60×) were used in the investigations. In this way some of the difficulties of Hanzon's method were eliminated: it was no longer necessary to fix the cover slip when working with water-immersion objectives, the intensity of illumination of the object examined was increased, the area of irrigation of the organ was increased yet virtually complete immobilization was provided. One of the special features of the contact objectives is the provision for ocular focusing. This makes it impossible to obtain serial photographs by the use of an ordinary camera. A special holder was suggested for the camera, enabling its

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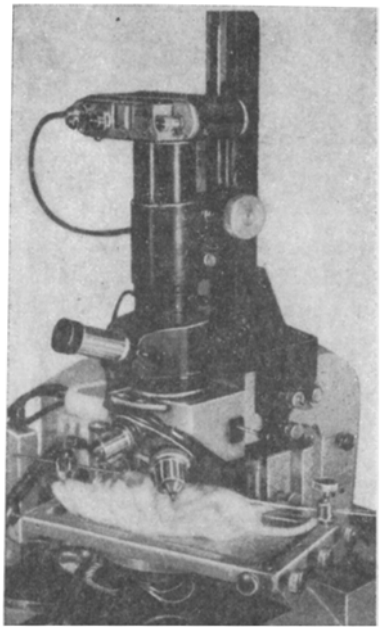

Fig. 1.

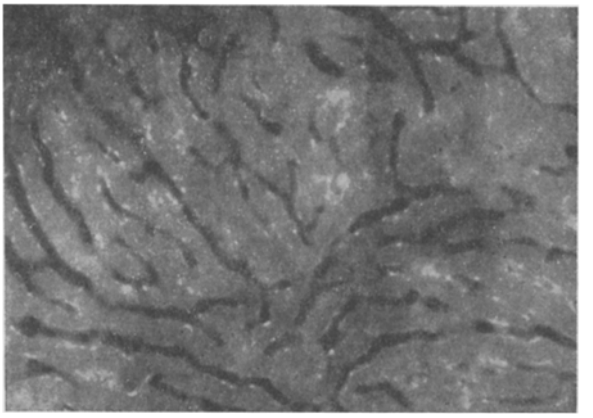

Fig. 2.

Fig. 1. General view of apparatus for intravital luminescence microscopy of the liver.

Fig. 2. Distribution of bovine globulin, labeled with fluorescein isocyanate, in the rat liver in the normal state in vivo (ob. 25, oc. 3).

position to be changed along the optical axis of the instrument (Fig. 1). The holder consists of a pedestal fixed in the desired position directly to the upper member of the microscope base. It is fitted with a controlling strip with toothed rack along which the tube-holder of the camera moves. The space between the outlet of the microscope and inlet of the camera is screened by means of a bellows. Work with the microscope is carried out in incident light.

The unit for fixing the animal consists of an electrically heated microscope stage of special design, easily mounted on the microscope in place of the usual one (Fig. 1). The stage is fitted with a nichrome heater powered from the main supply system through a step-down transformer. The temperature is controlled by a simple relay circuit and contact thermometer mounted directly in the stage. In this way the conditions of heating of the animal can be controlled reliably at will. The animal is fixed to a manipulation table made of stainless steel (Fig. 1). Around the perimeter of this table is a series of closed grooves with holes for draining the irrigation fluid. In addition, at the edges of the table special fixing sockets are provided for securing the various instruments in the desired position relative to the animal. The metal stage for the animal can be heated to a required temperature by direct contact with the electric heater of the microscope stage.

The irrigation unit consists of a vessel containing Tyrode fluid, a heating coil and ultrathermostat for maintaining the fluid at constant temperature, and a flexible tube ending in a needle for carrying the fluid to the organ to be studied. The temperature of the irrigating fluid (38°) is checked by an electrothermometer placed in the immediate neighborhood of the test organ, and it can be controlled by changing the operating conditions of the ultrathermostat.

The method of work with an animal consists of several stages, as follows. The animal is anesthetized by intraperitoneal injection of 20% urethane solution in a dose of 100 mg/100 g body weight, and placed on the metal table. Laparotomy is performed through a curved incision at the costal margin, the tip of the xiphisternum is removed, and the falciform ligament divided. Next, to minimize movements of the liver, dependent on respiratory movements of the diaphragm, a special speculum, fixed to a holder on the table for the animal, is introduced between the liver and diaphragm. A glass spatula, covered with moist gauze, is carefully inserted under the lobe of the liver to be investigated (usually the left lateral).

The spatula also is fixed to a holder on the table. To maintain the necessary humidity and temperature, the liver is irrigated throughout the experiment with Tyrode fluid. The whole exposed surface of the liver is packed with moist gauze towels. Only the very small area on which the contact objective is lowered remains free.

The animal stays in this position for 20-30 min until it recovers from the changes arising during preparation for the experiment. Microscopic investigation of the organ and photographic recording can then begin.

Biomicroscopy of the liver must begin with the study of autofluorescence of the organ. Next, depending on the objects of the investigation, various luminescent materials (uranine, protein labelled with fluorescein isothiocyanate) are injected into the animal's femoral vein and the permeability of the microvessels of the liver to the injected substances studied under normal and pathological conditions (Fig. 2).

The suggested modification of the method of intravital luminescence microscopy can be used with success to study the state not only of the liver, but also of other mammalian viscera.

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